explants were pre-incubated with the ALK5 inhibitor SB-505124 (50 μM) for 6 hours. Expression of MMP13, ALK5 and ALK1 was analyzed by quantitative PCR.

Results: Staining for both SMAD2/3P and SMAD1/5/8P was observed in human OA cartilage. Strikingly, SMAD2/3P staining was mostly negative in histological intact cartilage areas while cells in damaged areas, mainly in chondrocyte clusters, were often strongly positive. Also staining for SMAD1/5/8P was most intense in cells surrounding damaged regions and chondrocyte clusters. Incubation of human OA cartilage with TGF beta significantly down regulated MMP13 expression. This effect was totally abolished by the ALK5 inhibitor SB-505124. TGF beta also significantly elevated ALK5 expression, and decreased ALK1 expression, and both these effects were completely blocked by pre-incubation with SB-505124.

Conclusion: Stimulation of human end-stage OA cartilage with high TGF beta concentrations down regulates MMP13 expression and modulates TGF beta type I receptors, increasing the ALK5/ALK1 ratio. These effects all run via SMAD2/3 (ALK5). We propose, based on these results and earlier findings, that during aging TGF beta loses its protective role by a loss of SMAD2/3 signaling in articular chondrocytes. This loss plays a role in the initiation of cartilage degradation. In end-stage OA cartilage the majority of cells does not display either SMAD2/3P or SMAD1/5/8P. However, neighboring severely damaged cartilage a population of chondrocytes is present, most probably involved in (unsuccessful) repair, that expresses SMAD2/3P and SMAD1/5/8P.

211
MOLECULAR DETECTION OF A PRECURSOR STAGE OF OSTEOARTHRITIS IN RNA FROM CARTILAGE OF YOUNG ADULTS WITH HIP PAIN

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Purpose: Developmental dysplasia of hip (DDH) is recognized as a potential risk factor for hip pain in young adults and a risk factor for development of hip osteoarthritis (OA). Recently, femoroacetabular impingement (FAI), with other dysmorphic and orientation abnormalities of the hip joint, has been reported. Both conditions were presumed to be in an early stage of degeneration based on their long term history and radiographic evaluations, however, there are no reports of a biological basis for early OA or discrimination of DDH and FAI. Therefore, the purpose of this study was to determine the metabolic profile of the cartilage at the time of hip surgery to reshape the hip.

Methods: Fifty nine cartilage samples were obtained from patients who had FAI (n = 26), DDH (n = 13), and structural instability with impingement (combined) (n = 20), at the time of their joint preservation surgery, either surgical dislocation or/and periacetabular osteotomy. Cartilage samples from 2 children were used as normal control, and 7 OA samples were used as disease controls. All cartilage samples were harvested from their femoral neck junctions, and RNAs were extracted. These cartilage samples were evaluated by real-time PCR assay of gene expression of cytokines and chemokines, matrix molecules, and degradative enzymes. The macroscopic cartilage damage was classified by Beck's criteria (normal, malacia, cleavage, and defect).

Results: RNA from both diseases presented high levels of specific inflammatory cytokines (IL-1β and IL-8), chemokines (CXCL2, CXCL3, CXCL6, CCL3 and CCL3L1), and degenerative mediators (MMP-13 and ADAMTS-4), and this expression was higher in FAI than DDH or combined. In addition, expression of Type II collagen (Col2A1) and Aggrecan were higher in FAI compared to DDH and combined samples (Figure 1).

Stratification by age (<20 y.o., ≥20 y.o.) showed that older DDH and combined samples expressed higher levels of some chemokines (CCL3L1 in DDH, CXCL1 and CXCL2 in combined) and catabolic enzymes (MMP-13 in DDH, ADAMTS-4 in combined). In addition, the Col2A1 level was lower in older samples (Figure 2).

Comparison between Beck's classification showed a higher expression of a specific cytokine (IL-8), chemokines (CXCL1, CXCL3, CXCL6, and CCL3L1), and ADAMTS-4 in samples with cartilage cleavage. Col2A1 expression was gradually increased with advancing cartilage damage, while less expression in OA samples.

Conclusions: The cartilage condition in both diseases is considered pre-OA, based on the lack of radiographic OA. The cartilage of FAI has a high level of metabolic activity with more arthritic character (high catabolic factors) but also a regenerative response (high Col2A1 and Aggrecan). In both DDH and combined patients, the overall level of metabolic activity was lower than FAI, however the expression of degenerative enzymes was age-dependent indicating cartilage degradation especially in older patients. Therefore, both age and OA-related symptoms need to be taken into account when considering surgical intervention. We hypothesize that joint preservation surgery would decrease stress on the articular cartilage and lower metabolic activity. In conclusion, we have demonstrated that hip cartilage from young patients with FAI and DDH has a unique biological character, which may lead to identification of specific biomarkers for detection of early degenerative changes and outcomes following treatment.

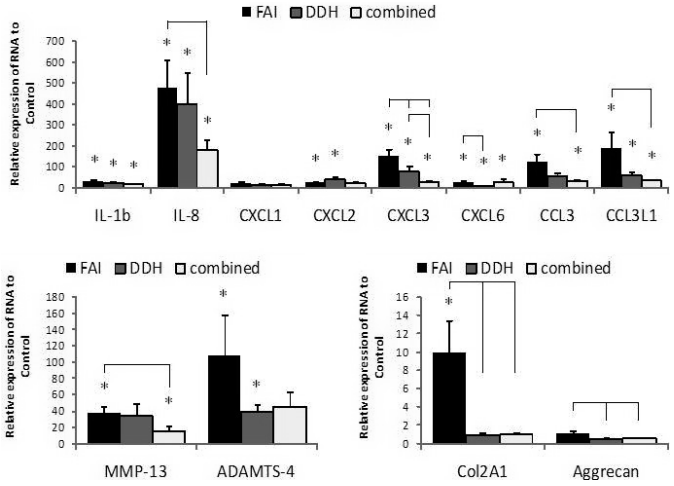

Fig. 1. Expression levels of cytokines, matrix molecules, and chemokines in FAI, DDH and combined samples. RNA expression was normalized to GAPDH mRNA and then compared with normal cartilage samples (set at 1). *Significant difference between normal control.

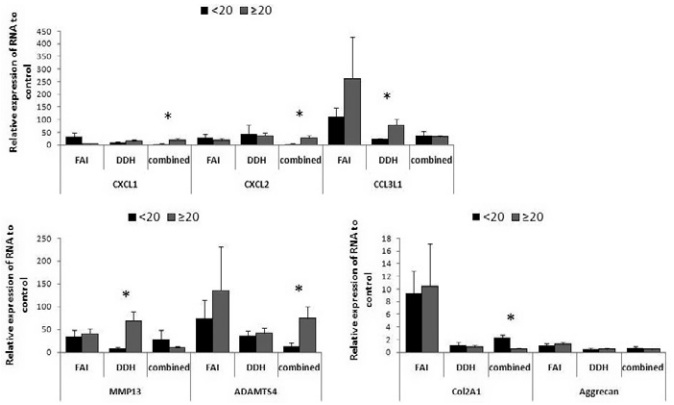

Fig. 2. Stratification by age (<20 y.o., ≥20 y.o.) in each disease. RNA expression was normalized to GAPDH mRNA and then compared with normal cartilage samples (set at 1). *Significant difference between each age group.

212
EVALUATION OF THE EFFICACY OF IL-1R ANTAGONISTS IN HUMAN CARTILAGE DEGRADATION *EX VIVO*

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Purpose: Osteoarthritis is the most common chronic joint disorder in the US, affecting nearly one-tenth of the population. It is characterized by degeneration of the articular cartilage, limited intra-articular inflammation and synovitis, changes in sub-chondral bone and pain. Nothing in the current standard of care preserves cartilage or carries a claim of disease modification in osteoarthritis; standard treatments focus on symptom relief using NSAIDs, injected steroids, and viscosupplements. IL-1, produced by chondrocytes in OA cartilage, promotes increased expression and activation of aggrecanases and matrix metalloproteinases